

Sequence Listing

<110> Ajinomoto Co. Inc.

5 <120> Method of constructing amino acid producing bacteria, and method of preparing amino acids by fermentation with the constructed amino acid producing bacteria

<130> OP 99052

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<150> JP 10-271786

<151> 1998-9-25

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<160> 6

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<211> 46

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<211> 46

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<400> 2

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<210> 3

<211> 46

<212> nucleic acid

<400> 3

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<211> 46

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<211> 46

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<220> primer A for cloning of gltA from Brevibacterium lactofermentum

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<210> 8

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 <220> primer 1 for introducing a mutation of gltA promoter

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 <220> primer 2 for introducing a mutation of gltA promoter

20 <400> 10
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25 <211> 40
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 <220> primer 4 for introducing a mutation of gltA promoter

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35 <220> gltA promoter sequence primer

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5 <211> 17

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<400> 23

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20 <400> 24

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<220> primer B for amplifying of nptII

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gcccgggggtg ggcgaagaac tcc

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 10 <212> nucleic acid
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<210> 28
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LA

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<210> 31

<211> 2766

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<213> *Brevibacterium lactofermentum* ATCC13869

<220> *Brevibacterium lactofermentum* ATCC13869 *pdhA gene* LA

<400> 31

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Met Ala Asp Gln Ala Lys Leu Gly Gly Lys Pro Ser Asp Asp Ser Asn

1

5

10

15

20 ttc gcg atg atc cgc gat ggc gtg gca tct tat ttg aac gac tca gat 96

Phe Ala Met Ile Arg Asp Gly Val Ala Ser Tyr Leu Asn Asp Ser Asp

20

25

30

ccg gag gag acc aac gag tgg atg gat tca ctc gac gga tta ctc cag 144

25 Pro Glu Glu Thr Asn Glu Trp Met Asp Ser Leu Asp Gly Leu Leu Gln

35

40

45

gag tct tct cca gaa cgt gct cgt tac ctc atg ctt cgt ttg ctt gag 192

Glu Ser Ser Pro Glu Arg Ala Arg Tyr Leu Met Leu Arg Leu Leu Glu

30

50

55

60

cgt gca tct gca aag cgc gta tct ctt ccc cca atg acg tca acc gac 240

Arg Ala Ser Ala Lys Arg Val Ser Leu Pro Pro Met Thr Ser Thr Asp

65

70

75

80

35

tac gtc aac acc att cca acc tct atg gaa cct gaa ttc cca ggc gat 288

Tyr Val Asn Thr Ile Pro Thr Ser Met Glu Pro Glu Phe Pro Gly Asp
 85 90 95
 gag gaa atg gag aag cgt tac cgt cgt tgg att cgc tgg aac gca gcc 336
 5 Glu Glu Met Glu Lys Arg Tyr Arg Arg Trp Ile Arg Trp Asn Ala Ala
 100 105 110
 atc atg gtt cac cgc gct cag cga cca ggc atc ggc gtc ggc gga cac 384
 Ile Met Val His Arg Ala Gln Arg Pro Gly Ile Gly Val Gly Gly His
 10 115 120 125
 att tcc act tac gca ggc gca gcc cct ctg tac gaa gtt ggc ttc aac 432
 Ile Ser Thr Tyr Ala Gly Ala Ala Pro Leu Tyr Glu Val Gly Phe Asn
 130 135 140
 15 cac ttc ttc cgc ggc aag gat cac cca ggc ggc ggc gac cag atc ttc 480
 His Phe Phe Arg Gly Lys Asp His Pro Gly Gly Gly Asp Gln Ile Phe
 145 150 155 160
 20 ttc cag ggc cac gca tca cca ggt atg tac gca cgt gca ttc atg gag 528
 Phe Gln Gly His Ala Ser Pro Gly Met Tyr Ala Arg Ala Phe Met Glu
 165 170 175
 ggt cgc ctt tct gaa gac gat ctc gat ggc ttc cgt cag gaa gtt tcc 576
 25 Gly Arg Leu Ser Glu Asp Asp Leu Asp Gly Phe Arg Gln Glu Val Ser
 180 185 190
 cgt gag cag ggt ggc att ccg tcc tac cct cac cca cac ggt atg aag 624
 Arg Glu Gln Gly Gly Ile Pro Ser Tyr Pro His Pro His Gly Met Lys
 30 195 200 205
 gac ttc tgg gag ttc cca act gtg tcc atg ggt ctt ggc cca atg gat 672
 Asp Phe Trp Glu Phe Pro Thr Val Ser Met Gly Leu Gly Pro Met Asp
 210 215 220
 35 gcc att tac cag gca cgt ttc aac cgc tac ctc gaa aac cgt ggc atc 720

Ala Ile Tyr Gln Ala Arg Phe Asn Arg Tyr Leu Glu Asn Arg Gly Ile
 225 230 235 240

aag gac acc tct gac cag cac gtc tgg gcc ttc ctt ggc gac ggc gaa 768
 5 Lys Asp Thr Ser Asp Gln His Val Trp Ala Phe Leu Gly Asp Gly Glu
 245 250 255

atg gac gag cca gaa tca cgt ggt ctc atc cag cag gct gca ctg aac 816
 Met Asp Glu Pro Glu Ser Arg Gly Leu Ile Gln Gln Ala Ala Leu Asn
 10 260 265 270
 aac ctg gac aac ctg acc ttc gtg gtt aac tgc aac ctg cag cgt ctc 864
 Asn Leu Asp Asn Leu Thr Phe Val Val Asn Cys Asn Leu Gln Arg Leu
 275 280 285

gac gga cct gtc cgc ggt aac acc aag atc atc cag gaa ctc gag tcc 912
 15 Asp Gly Pro Val Arg Gly Asn Thr Lys Ile Ile Gln Glu Leu Glu Ser
 290 295 300

ttc ttc cgt ggc gca ggc tgg tct gtg atc aag gtt gtt tgg ggt cgc 960
 20 Phe Phe Arg Gly Ala Gly Trp Ser Val Ile Lys Val Val Trp Gly Arg
 305 310 315 320

gag tgg gat gaa ctt ctg gag aag gac cag gat ggt gca ctt gtt gag 1008
 Glu Trp Asp Glu Leu Leu Glu Lys Asp Gln Asp Gly Ala Leu Val Glu
 25 325 330 335

atc atg aac aac acc tcc gat ggt gac tac cag acc ttc aag gct aac 1056
 Ile Met Asn Asn Thr Ser Asp Gly Asp Tyr Gln Thr Phe Lys Ala Asn
 340 345 350

gac ggc gca tat gtt cgt gag cac ttc ttc gga cgt gac cca cgc acc 1104
 30 Asp Gly Ala Tyr Val Arg Glu His Phe Phe Gly Arg Asp Pro Arg Thr
 355 360 365

gca aag ctc gtt gag aac atg acc gac gaa gaa atc tgg aag ctg cca 1152
 35 Ala Lys Leu Val Glu Asn Met Thr Asp Glu Glu Ile Trp Lys Leu Pro

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|----|--|-----|---------|
| | 515 | 520 | 525 |
| | acc ttc aag gaa ctg atg cgc gat aag ggc ttg gct gat cgc ctt gtc 1632 | | |
| | Thr Phe Lys Glu Leu Met Arg Asp Lys Gly Leu Ala Asp Arg Leu Val | | |
| 5 | 530 | 535 | 540 |
| | cca atc att cct gat gag gca cgt acc ttc ggt ctt gac tct tgg ttc 1680 | | |
| | Pro Ile Ile Pro Asp Glu Ala Arg Thr Phe Gly Leu Asp Ser Trp Phe | | |
| | 545 | 550 | 555 560 |
| 10 | cca acc ttg aag atc tac aac ccg cac ggt cag aac tac gtg cct gtt 1728 | | |
| | Pro Thr Leu Lys Ile Tyr Asn Pro His Gly Gln Asn Tyr Val Pro Val | | |
| | 565 | 570 | 575 |
| 15 | gac cac gac ctg atg ctc tcc tac cgt gag gca cct gaa gga cag atc 1776 | | |
| | Asp His Asp Leu Met Leu Ser Tyr Arg Glu Ala Pro Glu Gly Gln Ile | | |
| | 580 | 585 | 590 |
| | ctg cac gaa ggc atc aac gag gct ggt tcc gtg gca tcg ttc atc gct 1824 | | |
| 20 | Leu His Glu Gly Ile Asn Glu Ala Gly Ser Val Ala Ser Phe Ile Ala | | |
| | 595 | 600 | 605 |
| | gcg ggt acc tcc tac gcc acc cac ggc aag gcc atg att ccg ctg tac 1872 | | |
| | Ala Gly Thr Ser Tyr Ala Thr His Gly Lys Ala Met Ile Pro Leu Tyr | | |
| 25 | 610 | 615 | 620 |
| | atc ttc tac tcg atg ttc gga ttc cag cgc acc ggt gac tcc atc tgg 1920 | | |
| | Ile Phe Tyr Ser Met Phe Gly Phe Gln Arg Thr Gly Asp Ser Ile Trp | | |
| | 625 | 630 | 635 640 |
| 30 | gca gca gcc gat cag atg gca cgt ggc ttc ctc ttg ggc gct acc gca 1968 | | |
| | Ala Ala Ala Asp Gln Met Ala Arg Gly Phe Leu Leu Gly Ala Thr Ala | | |
| | 645 | 650 | 655 |
| 35 | ggt cgc acc acc ctg acc ggt gaa ggc ctc cag cac atg gat gga cac 2016 | | |
| | Gly Arg Thr Thr Leu Thr Gly Glu Gly Leu Gln His Met Asp Gly His | | |

| | | | | |
|----|--|-----|-----|-----|
| | 805 | 810 | 815 | |
| | ttc gta acc acc cag ctg aag cag acc tcc ggc cca tac gtt gca gtg 2496 | | | |
| | Phe Val Thr Thr Gln Leu Lys Gln Thr Ser Gly Pro Tyr Val Ala Val | | | |
| 5 | 820 | 825 | 830 | |
| | tct gac ttc tcc act gat ctg cca aac cag atc cgt gaa tgg gtc cca 2544 | | | |
| | Ser Asp Phe Ser Thr Asp Leu Pro Asn Gln Ile Arg Glu Trp Val Pro | | | |
| | 835 | 840 | 845 | |
| 10 | ggc gac tac acc gtt ctc ggt gca gat ggc ttc ggt ttc tct gat acc 2592 | | | |
| | Gly Asp Tyr Thr Val Leu Gly Ala Asp Gly Phe Gly Phe Ser Asp Thr | | | |
| | 850 | 855 | 860 | |
| 15 | cgc cca gct gct cgt cgc ttc ttc aac atc gac gct gag tcc att gtt 2640 | | | |
| | Arg Pro Ala Ala Arg Arg Phe Phe Asn Ile Asp Ala Glu Ser Ile Val | | | |
| | 865 | 870 | 875 | 880 |
| 20 | gtt gca gtg ctg aac tcc ctg gca cgc gaa ggc aag atc gac gtc tcc 2688 | | | |
| | Val Ala Val Leu Asn Ser Leu Ala Arg Glu Gly Lys Ile Asp Val Ser | | | |
| | 885 | 890 | 895 | |
| 25 | 900 | 905 | 910 | |
| | gtt tcc gta gat cca aac gct cct gag gaa 2766 | | | |
| | Val Ser Val Asp Pro Asn Ala Pro Glu Glu | | | |

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<210> 32

<211> 8556

<212> nucleic acid

<213> Brevibacterium lactofermentum ATCC13869

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| 5 | tcattgccact atcttgggggt tctcgggtatt agatcttctg ataaaaaccc gatagttttc | 180 |
| | ttgcgctaga cactaattac ggcaccgctt aagcatggtc gtgacacgta aaacctgact | 240 |
| | tagggcattt tgatgtgggtg tagatcatat tgacgtcaat gaatgaagtg actaactccg | 300 |
| | ccgaatccac atcgtctaaa aggcctgggc gaccacgtaa agacgggcac gacgagaaga | 360 |
| | tcattcgacgc aactttacgg ctcatcgaca gcaatcgccc cgctacggtc aatgcagttg | 420 |
| 10 | tcaaagaaag cggagtggca cgtgcagcgg ttatctgacg ctggcccagg ctagtggatc | 480 |
| | tagtagcggg agcttttagat gccgggcgag ctccagttga aatagatacc ccaggggaca | 540 |
| | tcaaagagac cttgattgat gggctgttta caaatcaggc gaaaaccact ggagtctcct | 600 |
| | atcctcgtca gcgatttcgc aaacggctcg agttgggtgat gtcagatcaa gaattacagc | 660 |
| | tcgcctaata gaattcacat gtgaagagac gtcgagaagc aaatatctgc gcgctgcaag | 720 |
| 15 | tcgcgcaaga aaaaggccaa atccgggcgg atctagacat cgaggcgtgc ctgatgcaa | 780 |
| | tccttgggggt gttttattac caatcggctg cgcgtggagt aaatttcacc gaccaaggta | 840 |
| | caacgcaacg atgcagagaa gccttgaggg tgatctggca tggaatggaa ccttaaattc | 900 |
| | aggttctgac gaggtgcgaa gcaagtgtgc gcgcgcgcga cctcagtatc cggatcaact | 960 |
| | taatttcgaa gtgctggggt ttctcgcgca tacccaatgc gtaccgatgt gcccatgagc | 1020 |
| 20 | gaaaaacagg ccacgataag ttcttataaa cttatcgtgg cctgcttcta tatttgtgcg | 1080 |
| | ccctgacggg ctggaaccgc cgacctgctg ggtgtaaacc agctgctctt ccagctgagc | 1140 |
| | taaaggcgcg cacgtgcttt tctagaacca ccttggtggc ctgaaagca acgagtgaaa | 1200 |
| | tactaacaca caatctccac agacctataa tcgctgctca ggccgtggaa attagcgatt | 1260 |
| | gttaaggctt cttgtttcca cgctggacga ggcaagaacc ttgccaatca ccgagacgtt | 1320 |
| 25 | ccgccttggt ctgcacgaga cctgccagtt gtgctgattc agagataact ccaggagcca | 1380 |
| | gggctccttc tttaaccaat ccaggagtca acaccagat acgaccattc tcagcgaggg | 1440 |
| | agcggatgga atccacaagt ccgtcgacga gatcgccgtc atcctcgcgc caccagagca | 1500 |
| | gcacgacatc gcacagctcg tcggtttctt catcgagtag ttctctaccg attgcatctt | 1560 |
| | cgatggactc gctgatcagc gtgtcggaat cttcatccca tccaatttct tgaacgatat | 1620 |
| 30 | gacccgattg aatgccgagt agttgagcat aatcctgggc accttgcttg actgcgcccg | 1680 |
| | gagcgtcggc cactttaata atcctcctcg tgtgggcccc gatgtgtttt tcgattacat | 1740 |
| | ggattcaaca tgaaaccgcg gggctattga tatatccgaa ttgcacatta ccgtccaacc | 1800 |
| | ggtactttga accacctttc cctggaattt ttctcttttc ctcccccttt acgtcaaga | 1860 |
| | atcaatgaat tcaatcactg gccagcgatt aacttttctg gttttcagtc ttggatttcc | 1920 |
| 35 | acaattctct tcaaaataat ggtggctaga tttttcatca aaccctcacc aaaaggacat | 1980 |
| | cagacctgta gttttatgcg attcgcgtca aacgtgagag aaacatcaca tctcacggga | 2040 |

ctt gac tct tgg ttc cca acc ttg aag atc tac aac ccg cac ggt cag 4072
 Leu Asp Ser Trp Phe Pro Thr Leu Lys Ile Tyr Asn Pro His Gly Gln

560 565 570

5 aac tac gtg cct gtt gac cac gac ctg atg ctc tcc tac cgt gag gca 4120
 Asn Tyr Val Pro Val Asp His Asp Leu Met Leu Ser Tyr Arg Glu Ala

575 580 585

10 cct gaa gga cag atc ctg cac gaa ggc atc aac gag gct ggt tcc gtg 4168
 Pro Glu Gly Gln Ile Leu His Glu Gly Ile Asn Glu Ala Gly Ser Val

590 595 600

15 gca tcg ttc atc gct gcg ggt acc tcc tac gcc acc cac ggc aag gcc 4216
 Ala Ser Phe Ile Ala Ala Gly Thr Ser Tyr Ala Thr His Gly Lys Ala

605 610 615

atg att ccg ctg tac atc ttc tac tcg atg ttc gga ttc cag cgc acc 4264
 Met Ile Pro Leu Tyr Ile Phe Tyr Ser Met Phe Gly Phe Gln Arg Thr

20 620 625 630 635

ggt gac tcc atc tgg gca gca gcc gat cag atg gca cgt ggc ttc ctc 4312
 Gly Asp Ser Ile Trp Ala Ala Ala Asp Gln Met Ala Arg Gly Phe Leu

640 645 650

25 ttg ggc gct acc gca ggt cgc acc acc ctg acc ggt gaa ggc ctc cag 4360
 Leu Gly Ala Thr Ala Gly Arg Thr Thr Leu Thr Gly Glu Gly Leu Gln

655 660 665

30 cac atg gat gga cac tcc cct gtc ttg gct tcc acc aac gag ggt gtc 4408
 His Met Asp Gly His Ser Pro Val Leu Ala Ser Thr Asn Glu Gly Val

670 675 680

35 gag acc tac gac cca tcc ttt gcg tac gag atc gca cac ctg gtt cac 4456
 Glu Thr Tyr Asp Pro Ser Phe Ala Tyr Glu Ile Ala His Leu Val His

685 690 695

cgt ggc atc gac cgc atg tac ggc cca ggc aag ggt gaa gat gtt atc 4504
 Arg Gly Ile Asp Arg Met Tyr Gly Pro Gly Lys Gly Glu Asp Val Ile
 700 705 710 715

5 tac tac atc acc atc tac aac gag cca acc cca cag cca gct gag cca 4552
 Tyr Tyr Ile Thr Ile Tyr Asn Glu Pro Thr Pro Gln Pro Ala Glu Pro
 720 725 730

10 gaa gga ctg gac gta gaa ggc ctg cac aag ggc atc tac ctc tac tcc 4600
 Glu Gly Leu Asp Val Glu Gly Leu His Lys Gly Ile Tyr Leu Tyr Ser
 735 740 745

15 cgc ggt gaa ggc acc ggc cat gag gca aac atc ttg gct tcc ggt gtt 4648
 Arg Gly Glu Gly Thr Gly His Glu Ala Asn Ile Leu Ala Ser Gly Val
 750 755 760

20 ggt atg cag tgg gct ctc aag gct gca tcc atc ctt gag gct gac tac 4696
 Gly Met Gln Trp Ala Leu Lys Ala Ala Ser Ile Leu Glu Ala Asp Tyr
 765 770 775

25 gga gtt cgt gcc aac att tac tcc gct act tct tgg gtt aac ttg gct 4744
 Gly Val Arg Ala Asn Ile Tyr Ser Ala Thr Ser Trp Val Asn Leu Ala
 780 785 790 795

30 cgc gat ggc gct gct cgt aac aag gca cag ctg cgc aac cca ggt gca 4792
 Arg Asp Gly Ala Ala Arg Asn Lys Ala Gln Leu Arg Asn Pro Gly Ala
 800 805 810

35 gat gct ggc gag gca ttc gta acc acc cag ctg aag cag acc tcc ggc 4840
 Asp Ala Gly Glu Ala Phe Val Thr Thr Gln Leu Lys Gln Thr Ser Gly
 815 820 825

cca tac gtt gca gtg tct gac ttc tcc act gat ctg cca aac cag atc 4888
 Pro Tyr Val Ala Val Ser Asp Phe Ser Thr Asp Leu Pro Asn Gln Ile
 830 835 840

| | | |
|----|--|------|
| | cgt gaa tgg gtc cca ggc gac tac acc gtt ctc ggt gca gat ggc ttc | 4936 |
| | Arg Glu Trp Val Pro Gly Asp Tyr Thr Val Leu Gly Ala Asp Gly Phe | |
| | 845 850 855 | |
| 5 | ggt ttc tct gat acc cgc cca gct gct cgt cgc ttc ttc aac atc gac | 4984 |
| | Gly Phe Ser Asp Thr Arg Pro Ala Ala Arg Arg Phe Phe Asn Ile Asp | |
| | 860 865 870 875 | |
| 10 | gct gag tcc att gtt gtt gca gtg ctg aac tcc ctg gca cgc gaa ggc | 5032 |
| | Ala Glu Ser Ile Val Val Ala Val Leu Asn Ser Leu Ala Arg Glu Gly | |
| | 880 885 890 | |
| 15 | aag atc gac gtc tcc gtt gct gct cag gct gct gag aag ttc aag ttg | 5080 |
| | Lys Ile Asp Val Ser Val Ala Ala Gln Ala Ala Glu Lys Phe Lys Leu | |
| | 895 900 905 | |
| 20 | gat gat cct acg agt gtt tcc gta gat cca aac gct cct gag gaa taaat | 5130 |
| | Asp Asp Pro Thr Ser Val Ser Val Asp Pro Asn Ala Pro Glu Glu | |
| | 910 915 920 | |
| 25 | cacctcaagg gacagataaa tcccgccgcc agacgttagt ctggcggcgg gattcgtcgt | 5190 |
| | aaagcaagct ctttttagcc gagaaacgcc ttgtcagaca atgttgccgc cttgatattg | 5250 |
| | gcgaactcct gcagcaaate gcgcacagtc aacttcgact tggtagcctg atctgcctgg | 5310 |
| | tagacaatct ggccttcacg catcatgac aggcgattgc ccaggcgaat tgectgttcc | 5370 |
| | atgttgctgcg tgaccataag cgtagtcaga gttccatctg ccacgatctt ttcggtcaag | 5430 |
| | gtggtcacaa gctctgcacg ctgtggatca agcgtctcgc tgtgctcacc caacagcatg | 5490 |
| | attttaggtt gagtaaaacc agccatcagc agggacaatg cctgacgctg accgccagag | 5550 |
| | agcaaaccac ctttggcagt gagcctgttt tccagacca gctcaaggcg ctcaagttcc | 5610 |
| 30 | tgcttgaatt gtcacggcg cttcgaggtc agtgcaaagc ccaatccacg gcgcttgccg | 5670 |
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